Project Report

# Summary

This report summarizes experiments conducted to evaluate and enhance the solubility of Allopregnanolone (Allo). The primary focus was to screen various organic solvents and assess the ability of numerous excipients to improve drug solubility, particularly in aqueous phosphate-buffered saline (PBS) following organic solvent removal.

Initial solvent screening identified Dichloromethane (DCM), Ethanol, and Isopropyl Alcohol as effective solvents for Allopregnanolone, while Acetone, HCl, and Citric Acid were unsuitable. Subsequent experiments evaluated a range of excipients, including Polysorbate 80, Span 20, Span 80, Tween 20, Phosphatidylcholine (PPTC), various PEGs, and oils, in combination with DCM or T-Butanol.

Despite extensive testing across various concentrations, pH levels, and excipient combinations, Allopregnanolone consistently precipitated. The drug "crashed out" of solution in nearly all formulations upon removal of the organic solvent and introduction of PBS, indicating a failure to achieve stable aqueous solubility with the tested components. Experimental challenges included material incompatibility between DCM and polystyrene microplates and process inconsistencies leading to variable solvent retention.

The key finding is that Allopregnanolone exhibits poor solubility and stability in the aqueous-based formulations investigated. The tested excipients and methods are insufficient for creating a stable solution. Further studies are required to explore alternative excipients, formulation strategies, and optimized solvent removal processes to address these solubility challenges.

# Introduction

Allopregnanolone is a compound with limited solubility, presenting challenges for its formulation. This study systematically evaluates methods to enhance its dissolution. A series of experiments determined the baseline solubility of Allopregnanolone in various solvents and assessed a range of excipients for their ability to improve solubility, particularly in aqueous buffer systems after the removal of organic solvents.

The investigation commenced with an initial screening of Allopregnanolone's solubility in several organic solvents, including Dichloromethane (DCM), T-Butanol, Ethanol, Isopropyl Alcohol, and Acetone, at concentrations ranging from 6.25 mg/mL to 100 mg/mL. Following this assessment, numerous excipients were evaluated for their potential as solubility enhancers, including surfactants (Polysorbate 80, Tween 20, Span 20, Span 80), phospholipids (Phosphatidylcholine), polymers (Dextran, PEG), amino acids, and oils. These excipients were tested in combination with Allopregnanolone in both DCM and T-Butanol.

The experimental approach involved preparing formulations of Allopregnanolone and selected excipients in organic solvents, typically within vials or 96-well microplates. These preparations underwent processing, such as continuous mixing or heating, to facilitate the removal of the organic solvent and assess the stability of Allopregnanolone in the remaining Phosphate Buffered Saline (PBS). Significant technical challenges arose during this process. Consistent precipitation of the drug, referred to as "crashing out," frequently occurred across most formulations. Additionally, procedural inconsistencies, including material splashing and caking during solvent evaporation and the dissolution of polystyrene plates by DCM, impacted the reproducibility of the results. This report details the methods and outcomes of these solubility enhancement attempts.

# Objectives

1. Determine the baseline solubility of Allopregnanolone in various organic solvents, including Dichloromethane (DCM), T-Butanol, Ethanol, and Isopropyl Alcohol, at multiple concentrations.

2. Evaluate the ability of various excipients (e.g., Phosphatidylcholine, Polysorbate 80, Span 20, and Tween 20) to enhance the solubility of Allopregnanolone in different solvent and buffer systems.

3. Assess the physical stability of Allopregnanolone formulations by identifying conditions, such as specific excipient combinations or pH levels, that result in drug precipitation.

# Methodology

## Materials

Allopregnanolone was the active pharmaceutical ingredient used in all experiments. The solvents utilized for solubility and formulation screening included dichloromethane (DCM), tert-butanol (T-Butanol), ethanol, isopropyl alcohol, and acetone. Aqueous solutions consisted of 1X phosphate-buffered saline (PBS), with specific experiments using PBS adjusted to pH 6.5, 7.5, and 10.0. A comprehensive library of excipients was evaluated, including Phosphatidylcholine (PPTC), Polysorbate 80 (P80), Span 20, Span 80, Tween 20, Tween 40, Castor Oil, Cholesterol, Corn Oil, Cottonseed Oil, Soybean Oil, L-Arginine, L-Cysteine, Glycine, L-Histidine, Dextran 40, Dextran 60-90, PEG 300, PEG 400, PEG 3350, Poloxamer 188, Soluplus, Dexolve, Beta-Cyclodextrin, and Gamma-Cyclodextrin.

## Preliminary Solvent Solubility Screening

The solubility of Allopregnanolone was determined in a range of solvents, including acetone, ethanol, isopropyl alcohol, and DCM. Pre-weighed amounts of Allopregnanolone (3.0–4.9 mg) were placed into individual glass vials. Solvents were added incrementally to achieve concentrations from 100 mg/mL to 6.25 mg/mL. After each solvent addition, the mixtures were mixed for 2 minutes, and solubility was assessed by visual inspection for the absence of solid particles. This procedure was conducted in accordance with internal standard operating procedure WID-001. The experimental parameters for this screening are detailed in `[TABLE\_1]`.

\*\*Table 1: Experimental parameters for the preliminary solubility assessment of Allopregnanolone in various solvents.\*\*

## Preparation of Stock Solutions

For formulation screening, stock solutions of Allopregnanolone and selected excipients were prepared in DCM, T-Butanol, or PBS. Allopregnanolone was typically dissolved at concentrations of 10 mg/mL or 12 mg/mL in DCM or T-Butanol. Excipient stock solutions were prepared at concentrations ranging from 0.625 mg/mL to 50 mg/mL, depending on the specific experiment and the solubility of the excipient in the chosen solvent system. The masses and volumes used to prepare representative stock solutions are provided in `[TABLE\_2]`.

\*\*Table 2: Mass and solvent volumes for the preparation of Allopregnanolone and excipient stock solutions.\*\*

## Excipient-Enhanced Solubility Assessment in Vials

The capacity of selected excipients to enhance Allopregnanolone solubility was evaluated in glass vials. Formulations were prepared by combining 1 mL of Allopregnanolone stock solution (10 mg/mL in DCM or T-Butanol) with 1 mL of an excipient stock solution (25 mg/mL in the corresponding solvent). Subsequently, 1 mL of 1X PBS was added to each vial. Control vials containing either drug-only or excipient-only solutions were also prepared. The organic solvent was removed from the vials via continuous mixing in a fume hood, after which the samples were visually inspected for drug precipitation. The appearance of these formulations in DCM and T-Butanol is shown in `[FIGURE\_1]` and `[FIGURE\_2]`, respectively.

\*\*Figure 1: Visual appearance of Allopregnanolone and excipient formulations in DCM and PBS.\*\*

\*\*Figure 2: Visual appearance of Allopregnanolone and excipient formulations in T-Butanol and PBS.\*\*

## High-Throughput Screening of Excipient Formulations

A high-throughput screening method using 96-well plates was employed to evaluate a broader range of excipients. Experiments were conducted in both Nunc (polystyrene) and Costar (polypropylene) plates. Test wells were prepared by combining Allopregnanolone and excipient stock solutions, typically 50 µL of each, followed by the addition of 100 µL of PBS. The plate layout included drug-only and excipient-only controls in duplicate or triplicate, as shown in the representative configuration in `[TABLE\_3]`. To remove the organic solvent, plates were heated to 70°C with gentle shaking for 15–32 minutes. Absorbance measurements were taken at 600 nm and 860 nm using a Tecan plate reader before and after the heating process. Final assessment of solubility was determined by visual inspection for drug precipitation after the plates had cooled `[FIGURE\_3]`. Due to the observed dissolution of polystyrene by DCM, subsequent experiments involving this solvent were performed exclusively in polypropylene plates `[FIGURE\_4]`.

\*\*Table 3: Representative 96-well plate configuration for screening T-Butanol-based excipient formulations.\*\*

\*\*Figure 3: Nunc polystyrene plate wells containing DCM and T-Butanol formulations after solvent evaporation at 70°C.\*\*

\*\*Figure 4: Costar polypropylene plate wells containing DCM formulations before and after solvent evaporation at 70°C.\*\*

# Results

## Initial Allopregnanolone Solvent Solubility Screening

The solubility of Allopregnanolone (Allo) was evaluated in a panel of organic solvents at concentrations ranging from 100 mg/mL to 6.25 mg/mL. Complete dissolution was achieved in Dichloromethane (DCM) at 100 mg/mL and in Tert-Butanol (T-Butanol) at 25 mg/mL. In Ethanol and Isopropyl Alcohol, Allo was soluble at 6.25 mg/mL but not at higher concentrations (12.5, 25, 50, and 100 mg/mL). Allopregnanolone did not dissolve in Acetone, HCl, or Citric Acid at any tested concentration down to 6.25 mg/mL. A summary of the determined solubilities is provided in Table 1.

| Solvent | Maximum Observed Solubility (mg/mL) | Dissolution |

| :--- | :--- | :--- |

| Dichloromethane (DCM) | 100 | Yes |

| Tert-Butanol | 25 | Yes |

| Ethanol | 6.25 | Yes |

| Isopropyl Alcohol | 6.25 | Yes |

| Acetone | 6.25 | No |

| HCl | 6.25 | No |

| Citric Acid | 6.25 | No |

## Evaluation of Excipients in Dichloromethane and Tert-Butanol using Glass Vials

The capacity of four excipients—Phosphatidylcholine (PPTC), Polysorbate 80 (P80), Span 80, and Tween 20—to maintain Allopregnanolone solubility in an aqueous phase was assessed in glass vials. Stock solutions of Allo (10 mg/mL) and excipients (25 mg/mL) were prepared in either DCM or T-Butanol, combined, and mixed with 1X Phosphate-Buffered Saline (PBS). Visual inspection of the resulting mixtures in DCM and T-Butanol is shown in Figures 1 and 2. During the organic solvent removal via continuous mixing, significant splashing and caking of material were observed on the vial walls, resulting in inconsistent final PBS volumes across samples.

## Excipient Screening in Polystyrene and Polypropylene Microplates

Screening was transitioned to 96-well microplates to improve throughput. Formulations containing Allo (10 mg/mL) and excipients (PPTC, P80, Span 80, Tween 20 at 25 mg/mL) in either DCM or T-Butanol were prepared in individual wells, followed by the addition of PBS and heating at 70°C to evaporate the organic solvent.

Initial experiments in Nunc polystyrene plates revealed material incompatibility. Wells containing DCM exhibited dissolution of the polystyrene upon heating for 15 minutes, compromising the integrity of the experiment (Figures 3 and 4). In the T-Butanol cohort using the same Nunc plates, the plate material remained intact, but all tested excipients failed to prevent Allo from precipitating out of solution upon solvent evaporation (Figures 5 and 6). Before evaporation, P80 and Tween 20 appeared soluble after the addition of PBS, while Span 80 was not soluble on its own.

The DCM-based experiment was repeated using Costar polypropylene plates, which are resistant to the solvent. After heating at 70°C for 15 minutes to evaporate the DCM, a small amount of precipitate was observed in all wells containing Allo and an excipient (Figures 7 and 8).

## Comprehensive Screening of Excipient Formulations

An expanded screening effort evaluated a wider range of excipients and formulation conditions for their ability to solubilize Allopregnanolone in PBS after solvent evaporation. Excipients were categorized based on their solubility in PBS, T-Butanol, or DCM, with stock concentrations prepared as detailed in Table 2. Experiments involved combining Allo stock solutions (12 mg/mL in DCM or T-Butanol) with excipient solutions, adding PBS, and heating at 70°C to remove the organic solvent.

| Solvent System | Excipient | Concentration (mg/mL) |

| :--- | :--- | :--- |

| PBS Soluble | L-Arginine, L-Cysteine, Dextran 40, Dextran 60-90, Glycine, PEG 300, PEG 3350, PEG 400 | 50 |

| | L-Histidine, Beta-Cyclodextrin, Gamma-Cyclodextrin | 10 |

| | Poloxamer 188, Polysorbate 80, Tween 20, Dexolve | 25 |

| | Soluplus, Span 20 | 6.25 |

| | Tween 40 | 12.5 |

| T-Butanol Soluble | Castor Oil, Corn Oil, Cottonseed Oil, Soybean Oil | 25 |

| | PEG 300, PEG 400 | 50 |

| | Cholesterol | 0.625 |

| | PPTC, Polysorbate 80, Span 20, Tween 20, Tween 40 | 10 |

| DCM Soluble | Castor Oil, Corn Oil, Cottonseed Oil, Soybean Oil | 50 |

| | Cholesterol | 20 |

| | PPTC, Polysorbate 80, Span 20, Tween 20, Tween 40 | 10 |

In a targeted study, oil-based excipients (Castor Oil, Corn Oil, Cottonseed Oil) at a final concentration of 25 mg/mL were combined with Allo (6 mg/mL) in DCM. Following a 14-minute heating period at 70°C to remove DCM, Allo was observed to have precipitated in all samples (Table 3).

| Excipient (25 mg/mL) | Allo Conc. (mg/mL) | Heating | Observation |

| :--- | :--- | :--- | :--- |

| Castor Oil | 6 | 14 min @ 70°C | Allo crashed out |

| Corn Oil | 6 | 14 min @ 70°C | Allo crashed out |

| Cottonseed Oil | 6 | 14 min @ 70°C | Allo crashed out |

Further tests focused on Span 20 and Span 80 at a fixed concentration (5 mg/mL) with varying final Allo concentrations of 3, 4, 5, and 6 mg/mL in DCM. In all conditions, Allo precipitated after solvent removal (Table 4). The effect of aqueous phase pH was also investigated by combining Allo (6 mg/mL) and Span 20 (5 mg/mL) with PBS at pH 6.5 and 10.0; precipitation occurred in both cases. Combinations of excipients (Span 20 with Tween 20 or Castor Oil) with Allo also resulted in precipitation.

| Excipient | Excipient Conc. (mg/mL) | Allo Conc. (mg/mL) | Observation |

| :--- | :--- | :--- | :--- |

| Span 20 | 5 | 3, 4, 5, 6 | Allo crashed out |

| Span 80 | 5 | 3, 4, 5, 6 | Allo crashed out |

Despite the observed precipitation, Allopregnanolone dissolved to some degree when mixed with Span 20 after DCM solvent removal via heating and evaporation. The amount of solubilized Allopregnanolone appeared consistent across the tested concentrations of 3, 4, 5, and 6 mg/mL.

\* \*\*Plate Material\*\* — DCM was incompatible with polystyrene plates under heating at 70°C, causing the wells to dissolve. Polypropylene plates were required for experiments involving DCM evaporation.

\* \*\*Solvent Evaporation\*\* — The transition from an organic solvent (DCM or T-Butanol) to an aqueous phase (PBS) upon heating was the critical step where Allopregnanolone precipitation consistently occurred across nearly all tested excipients.

\* \*\*Span 20\*\* — This was the only excipient noted to achieve some degree of Allopregnanolone dissolution in PBS after solvent removal, suggesting it may partially enhance aqueous solubility.

# Conclusion

The attempt to enhance the aqueous solubility of allopregnanolone in PBS using various excipients with DCM and T-butanol co-solvents yielded limited success. Initial experiments faced methodological issues, including material splashing and caking during solvent removal in vials, as well as the chemical incompatibility of polystyrene plates with DCM at elevated temperatures. These factors compromised the reproducibility and integrity of the results, necessitating a shift to more robust polypropylene plates for subsequent tests.

A broad screening of excipients—including phosphatidylcholine, polysorbate 80, Tween 20, and various oils—revealed that most were ineffective at preventing allopregnanolone from precipitating out of the aqueous phase following organic solvent evaporation. However, Span 20 was identified as the only excipient to demonstrate a noticeable solubilizing effect for allopregnanolone in PBS after the removal of DCM. This partial dissolution was consistent across a range of initial allopregnanolone concentrations from 3 to 6 mg/mL.

While a stable aqueous formulation of allopregnanolone was not achieved, these findings identified Span 20 as a promising candidate for future development. Further experimentation is required to quantify the exact solubility limit with Span 20 and to screen additional excipients using the refined experimental procedures.